Simultaneous measurement of peritoneal glucose and free water osmotic conductances

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Ultrafiltration (UF) failure is one of the most important causes of long-term peritoneal dialysis (PD) failure in patients. Osmotic forces acting across small and ultra-small pores generate a UF with solutes through the small pore and free water transport (FWT) through the ultra-small pore. The ability of glucose to exert an osmotic pressure sufficient to cause UF is the so-called 'osmotic conductance to glucose' (OCG) of the peritoneal membrane. Our study proposes a simple method to determine both the OCG and FWT. In 50 patients on PD, a Double Mini-Peritoneal Equilibration Test (Double Mini-PET), consisting of two Mini-PET, was performed consecutively. A solution of 1.36% glucose was used for the first test, whereas a solution of 3.86% glucose was used for the second test. The sodium removal values and the differences in UF between the two tests were used to calculate FWT and the OCG. Patients with UF failure showed significant reductions not only in the OCG and the FWT but also of UF of small pores. The Double Mini-PET is simple, fast, and could become useful to evaluate patients on PD in everyday clinical practice.

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Ultrafiltration (UF) across the peritoneal membrane is one of the major predictors of outcome and mortality in peritoneal dialysis (PD) patients.[1,2] UF failure (UFF) of peritoneal membrane is one of the most important causes of PD failure.[3–5] Peritoneal UF, in the early phase, is induced by the crystalloid osmotic pressure due to glucose. Later, the peritoneal UF is partially re-absorbed. The total amount of UF is mainly due to the early phase of a peritoneal dwell when the osmotic agent is glucose.[6] The effectiveness of the osmotic pressure, due to glucose, to generate UF is the so-called 'osmotic conductance to glucose' (OCG) of the peritoneal membrane.[7] According to the theory of three pores,[8–10] the osmotic forces, mainly acting across small pores (SP) and ultra-small pores (USP) (which have also been defined as aquaporins-1) in the peritoneal capillary wall, generate a UF with solutes through the SP (UFSP) and a UF without solutes or free water transport (FWT) through the USP.[11,12] On the basis of computer simulation[9] and experimental assessment,[13] the FWT was estimated to be approximately 50% of the peritoneal UF during an hypertonic (glucose 3.86%) dwell, despite the fact that a very small percentage ($\approx$2%) of the total pore area in the capillary wall was made up of USP.[9]

Some causes of UFF have been recognized: (1) high rates of small solute transport, (2) decreased OCG, (3) high rate of peritoneal absorption of dialysis fluid, and (4) small peritoneal surface area.[14]

The decreased OCG is associated with a markedly reduced sieving of sodium and it is commonly attributed to an impairment of the aquaporin-1 function.[5]

At present, only complicated peritoneal tests[7,15] or computer simulations[6] are available to assess OCG.

Recently, we showed a simple and fast method to assess the FWT by a peritoneal equilibration test (PET) with 3.86% glucose solution, lasting 1 h (Mini-PET), in a small group of PD patients [La Milia V *et al.*, *Nephrol Dial Transplant* 2002; **17**(Suppl 3):17–18 (abstract)]. The Mini-PET was sophisticated using an intraperitoneal volume marker and correcting for sodium diffusion, so that FWT could be calculated at every time point during a classical 3.86% PET lasting 4 h,[16] was validated by computer simulation suggesting also an algorithm of correction for lymphatic absorption and sodium diffusion[17] and applied in a larger group of PD patients.[13]

Furthermore the FWT, calculated by our method, was correlated to vascular aquaporin-1 expression.[18]

As demonstrated by Stelin and Rippe,[7] on the basis of the principle of 'osmotic transients,'[19] it is possible to assess the initial rate of fluid filtration (osmosis) occurring from the blood to the peritoneal cavity by means of two dwells with two different glucose concentrations (1.36 and 3.86%), shortening the dwell time to 1 h,[6] and without using intraperitoneal volume markers or computer-based calculations. Indeed, assuming that all parameters, but glucose concentration, remain constant between the two dwells, it is possible to assess OCG by the difference in initial UF rates (see Materials and Methods and Appendix).

The aim of this study was to propose a simple method to assess both OCG and FWT in PD patients. Moreover, the influence of sodium diffusion on the assessment of FWT was evaluated.

RESULTS

Table 1 summarizes the peritoneal transport characteristics during the Double Mini-PET.

As described elsewhere,[20–23] the values of dialysate/plasma creatinine concentration ratio ($D/P_{Creatinine}$) and mass transfer area coefficient (MTAC) of creatinine ($MTAC_{Creatinine}$) were similar when assessed with different glucose concentrations. As expected, UF, the ratio of dialysate glucose concentrations (D/D_0), the MTAC of glucose ($MTAC_{Glucose}$), the dialysate/sodium concentration ratio (D/P_{Na}), and the sodium removal (NaR) values of the two Mini-PETs were different.

The OCG was 3.28 ± 1.46 μl/min/mm Hg. According to OCG values, patients were categorized into four groups (Figure 1). Eight (16%) patients had an OCG > 4.74 μl/min/mm Hg, 12 (24%) patients had values of OCG between 3.30 and 4.74 μl/min/mm Hg, 22 (44%) patients had values of OCG between 1.82 and 3.29 μl/min/mm Hg, and 8 (16%) patients had an OCG < 1.82 μl/min/mm Hg.

No correlation between OCG and $MTAC_{Glucose}$ ($r^2 = 0.007$, $P = 0.5583$) and between OCG and $MTAC_{Creatinine}$ ($r^2 = 0.001$, $P = 0.7970$) was found.

The UFSP and FWT were 251 ± 133 and 243 ± 79 ml, respectively (Figure 2), and each contributed to approximately 50% of total UF.

The linear correlation coefficient between UF, during the classical 3.86%-PET (lasting 4 h), $MTAC_{Creatinine}$ and OCG was good but not very high ($r^2 = -0.15$, $P < 0.01$ and $r^2 = 0.16$, $P < 0.01$, respectively). The multiple linear correlation analysis between UF, during the classical 3.86%-PET, and both $MTAC_{Creatinine}$ and OCG, as covariates, showed a better correlation ($r^2 = 0.27$, $P < 0.01$).

The dialysate sodium concentration during the 1.36%-Mini-PET was analyzed to evaluate the sodium diffusion. During the 1.36%-Mini-PET, the so-called sodium sieving was observed, and this indicates the presence of FWT also during a dwell with a slightly hyperosmotic solution. The reduction of dialysate sodium concentration during the 1.36%-Mini-PET was 2.2 ± 2.2 mmol/l; the dialysate sodium concentration increased only in eight patients (mean increase $= 1.1 \pm 1.0$ mmol/l). After correcting for the convective transport, a positive amount of sodium diffusion during the 1.36%-Mini-PET was present only in five patients and the difference of UFSP (calculated with and without sodium diffusion) was 20 ml.

Table 1 | Peritoneal transport characteristics of 50 patients in PD therapy assessed during the Double Mini-PET

	1.36%-Mini-PET	3.86%-Mini-PET	*P*-value
UF (ml)	99 ± 95	494 ± 171	<0.0001
UFR (ml/min)	1.3 ± 1.3	6.6 ± 2.3	<0.0001
D/D_0	0.61 ± 0.06	0.51 ± 0.05	<0.0001
$MTAC_{Glucose}$ (ml/min)	15.2 ± 3.6	20.6 ± 3.6	<0.0001
$D/P_{Creatinine}$	0.39 ± 0.08	0.38 ± 0.08	0.0856
$MTAC_{Creatinine}$ (ml/min)	11.7 (10.8–16.1)	12.1 (9.9–16.5)	0.6894
D/P_{Na}	0.94 ± 0.02	0.88 ± 0.03	<0.0001
NaR (mmol)	8.0 (0.9–12.6)	34.9 (21.0–45.0)	<0.0001

D/D_0, ratio of dialysate glucose concentrations at end and at start of tests; $D/P_{Creatinine}$, dialysate/plasma creatinine concentration ratio; D/P_{Na}, dialysate/plasma sodium concentration ratio; $MTAC_{Glucose}$, mass transfer area coefficient of glucose; $MTAC_{Creatinine}$, mass transfer area coefficient of creatinine; NaR, sodium removal during the tests; PD, peritoneal dialysis; PET, Peritoneal Equilibration Test; UF, peritoneal ultrafiltration at end of tests; UFR, peritoneal ultrafiltration rate.
Data are expressed as means ± 1 s.d. or as median values and interquartile ranges in the parenthesis.

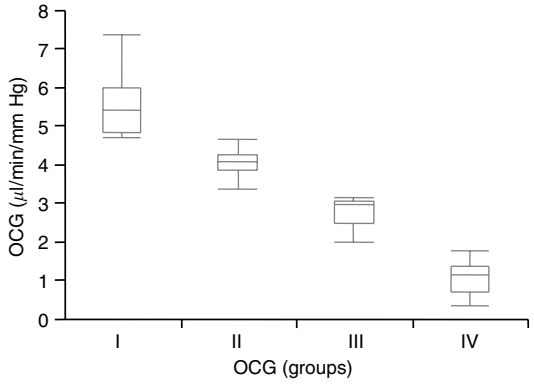

Figure 1 | Groups of patients according to osmotic conductance to glucose (OCG) assessed with the Double Mini-PET.

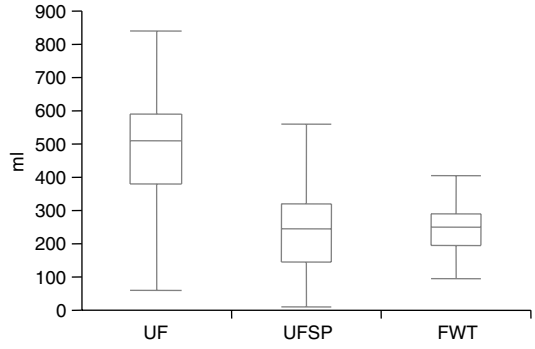

Figure 2 | UF, UFSP and FWT during the 3.86% test of Double Mini-PET. UF, ultrafiltration; UFSP, ultrafiltration through small pores; FWT, free water transport.

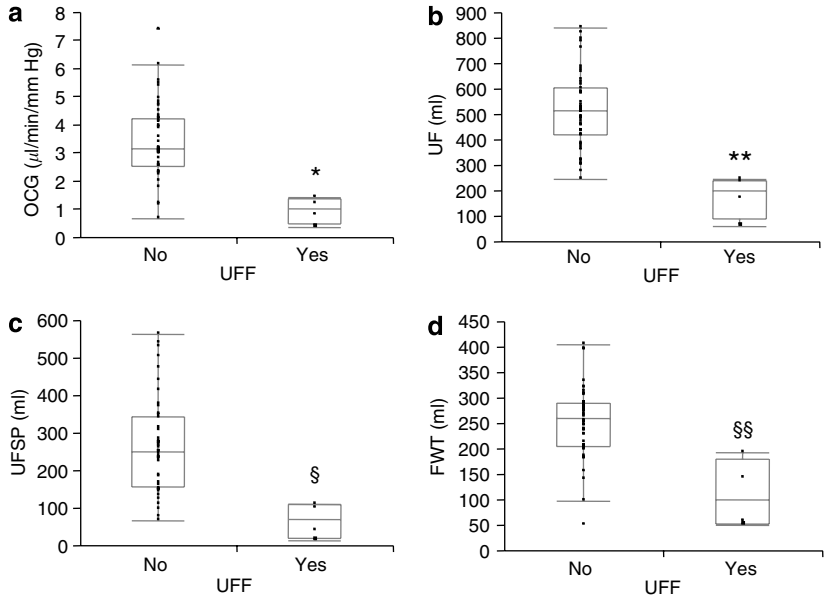

Figure 3 | Difference of (a) OCG, (b) UF, (c) UFSP and (d) FWT between the PD patients with and without UFF during the Double Mini-PET. OCG, osmotic conductance to glucose; UF, ultrafiltration; UFSP, ultrafiltration through small pores; FWT, free water transport; UFF, ultrafiltration failure. UF, UFSP, and FWT were calculated during the 3.86% test of Double Mini-PET. *$P = 0.0006$ vs no UFF; **$P < 0.0001$ vs no UFF; $^{\S}P = 0.0030$ vs no UFF; $^{\S\S}P = 0.0030$ vs no UFF.

In all patients, the UFSP and FWT after correction for sodium diffusion were nearly identical to UFSP and FWT calculated without sodium diffusion (249 ± 134 vs 251 ± 133 and 244 ± 76 vs 243 ± 79 ml, respectively).

To explore the possible clinical application of the Double Mini-PET, we analyzed the patients with UFF. Among the 50 patients, 4 had UFF according to the results of the previously performed classical 3.86%-PET lasting 4 h (i.e., total UF <400 ml after 4 h of 3.86%-PET). The only differences observed between the patients with UFF and the patients with normal peritoneal UF were the lower age (median age = 47 years, range = 27–55 years vs median age = 62 years, range = 31–82 years) and the longer time on PD treatment (median time = 42 months, range = 19–61 months vs median time = 5 months, range = 2–159 months) in UFF patients. During the 3.86%-Mini-PET, the UF was 180 ± 84 ml in the patients with UFF and 521 ± 147 ml ($P < 0.0001$) in the patients without UFF (Figure 3b). The OCG was 0.98 ± 0.48 μl/min/mm Hg in the patients with UFF and 3.48 ± 1.34 μl/min/mm Hg ($P = 0.0006$) in the patients without UFF (Figure 3a).

The patients with UFF showed not only a significant reduction of FWT but also a significant decrease of UFSP. The FWT was 112 ± 69 ml in the patients with UFF and 254 ± 70 ml ($P = 0.0030$) in the patients without UFF (Figure 3d); the UFSP was 68 ± 48 ml in the patients with UFF and 267 ± 126 ml ($P = 0.0030$) in the patients without UFF (Figure 3c). All the patients with UFF belonged to the group of patients with the lowest levels of OCG, but also some of the patients without UFF belonged to the same group; however, an OCG >1.81 μl/min/mm Hg was never associated with UFF (Figure 3a).

The D/D_0, $MTAC_{Glucose}$, $D/P_{Creatinine}$, and $MTAC_{Creatinine}$ values assessed during the Double Mini-PET were only slightly higher in the patients with UFF in comparison with the patients without UFF (Figure 4).

DISCUSSION

The possibility to estimate the characteristics of the transport through the peritoneal membrane could be a useful tool to explore the complex physiology of the peritoneal membrane. However, the tests to assess some of these mechanisms are extremely complex and they cannot be applied in everyday clinical evaluation of patients on PD. Until now, OCG was assessed only with complicated tests and complex calculations. We described previously the 3.86%-Mini-PET as a simple and fast method to assess the FWT in PD patients.[13]

This study is an extension of the study on 3.86%-Mini-PET and its aim is to propose a simple method to assess simultaneously FWT, OCG, and the classical parameters of peritoneal membrane small solute transport as D/D_0, D/P, and MTAC in PD patients. Moreover, the influence of sodium diffusion on the assessment of FWT was evaluated.

In this study, after performing a Double Mini-PET in 50 PD patients, we calculated the OCG and FWT with the only aid of a hand calculator. To our knowledge, this is the first simple test that allows one to assess the OCG, UFS, FWT, and the classical parameters of peritoneal small solute transport (D/D_0, D/P, and MTAC) simultaneously.

The analysis of the classical parameters of peritoneal small solute transport, assessed with the two tests (a 1.36%-Mini-PET and a 3.86%-Mini-PET) that compose the Double Mini-PET, showed that the results were similar to the findings of previous studies that had compared this transport during a

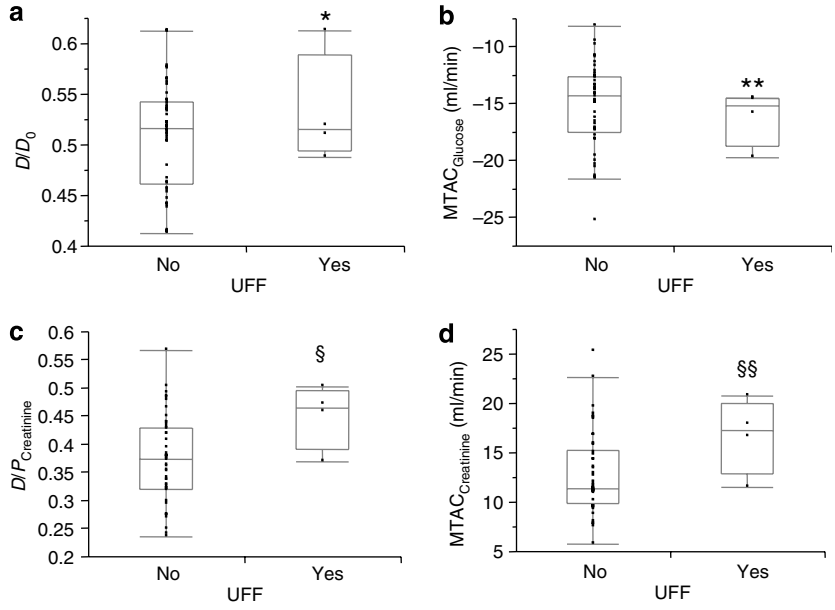

Figure 4 | Difference of D/D_0 (a), $MTAC_{Glucose}$ (b), $D/P_{Creatinine}$ (c) and $MTAC_{Creatinine}$ (d) between the PD patients with and without UFF during the Double Mini-PET. D/D_0, ratio of dialysate glucose concentrations; MTAC, mass transfer area coefficient; D/P, dialysate/plasma concentration ratio; UFF, ultrafiltration failure. D/D_0, D/P, and MTACs were calculated during the 1.36% test of Double Mini-PET: $^{*}P = 0.3643$ vs no UFF; $^{**}P = 0.3099$ vs no UFF; $^{\S}P = 0.1064$ vs no UFF; $^{\S\S}P = 0.0759$ vs no UFF.

PET lasting 4 h with different glucose concentrations in the fresh PD fluid.[20–23] Indeed, the $D/P_{Creatinine}$ and $MTAC_{Creatinine}$ were not different during the two tests with different glucose concentration. As expected, UF, D/P_{Na}, and the parameters of glucose transport (D/D_0 and $MTA_{CGlucose}$) were different with the two different solutions. These results indicate that the peritoneal transport of small solutes during a short PET and during a classical PET lasting 4 h is similar.

The OCG has been rarely assessed in PD patients, and it is not yet known if the reduction of OCG could be due to a reduced function of USPs (aquaporin-1 channels) or to a global reduction of PD membrane pores function or to other mechanisms.

Stelin and Rippe[7] found an mean OCG 3.54 ± 2.90 μl/min/mm Hg in 12 PD patients without UFF. This value is near to the mean value found in our patients (3.28 ± 1.46 μl/min/mm Hg). Waniewski *et al.*[15] reported a mean OCG 5.8 μl/min/mm Hg in patients without UFF and of 3.7–4.4 μl/min/mm Hg in patients with UFF, but the study was performed with an intraperitoneal volume marker and the method used was different from the method used in this study. Finally, the OCG values obtained with the Double Mini-PET were in the range of the values reported by computer simulations for the PD patients.[6]

The results of our study showed that the inclusion of both $MTAC_{Creatinine}$ and OCG, as covariates, increased the variance in UF capacity of peritoneal membrane (assessed by the classical 3.86%-PET) from 15 ($MTAC_{Creatinine}$ alone) to 27% ($MTAC_{Creatinine}$ and OCG). In conclusion, OCG could explain a relevant part of variability in the UF capacity of peritoneal membrane.

According to computer calculations[9] and as shown previously,[13] the USP and the FWT each contribute for approximately 50% to the total UF. One possible criticism to our method may be the absence of correction for sodium diffusion in the assessment of FWT;[16,17] however, the absence of correction for sodium diffusion has been demonstrated to result just in a slight underestimation of FWT.[17] It can be hypothesized that in patients with UFF, the diffusive transport of sodium could be higher than in patients without UFF, because in patients with UFF, the increase of MTAC of small solutes (and then also the MTAC of sodium) is frequent. It is difficult to assess the MTAC of sodium, as shown by several studies that reported values of MTAC of sodium much lower than the theoretical ones,[6] because there are small differences of sodium concentrations between plasma and dialysate. We were not able to calculate the MTAC of sodium in this study (not only during the 3.86%-Mini-PET but also during the 1.36%-Mini-PET); however, during the 1.36%-Mini-PET, we used the NaR, corrected for the convective transport, to correct the quantification of UFSP and FWT for sodium diffusion: UFSP and FWT were similar with and without the correction for sodium diffusion.

These data confirm that our method only slightly underestimated the quantification of FWT, also in patients with UFF (mean difference of 20 ml).

The correction for sodium diffusion could be important to quantify UFSP and FWT during the classical 3.86%-PET lasting 4 h; however, the correction for sodium diffusion should not influence the quantification of UFSP and FWT during the Mini-PET or the Double Mini-PET, because the diffusive transport of sodium is low during a very short

dwell. In any case, UFSP and FWT values could be corrected with a simple algorithm.[17]

In spite of the small number of patients with UFF in our study, we may deduce some interesting considerations about the mechanism of UFF. In all the patients with UFF, we found a marked reduction of OCG. The decrease of OCG was associated not only with a reduction of FWT but also of UFSP. In other words, in patients with UFF, the reduction of OCG was not a selective alteration of aquaporin-1, but was associated with a global reduction of UF capacity of SPs and USPs, that is the pores that contribute to UF formation under an osmotic pressure. This is in agreement with computer simulations suggesting that aquaporins are unlikely to be affected in marked UFF.[24,25] In contrast, Monquil *et al.*[26] examined a group of six PD patients with UFF that was attributed to an impairment of transcellular water transport or FWT because of the reduced difference in net UF obtained with a PET with glucose 1.36% and a PET with glucose 3.86% PD solutions. However, the difference in net UF that was obtained with the two PETs (with glucose 1.36% and with glucose 3.86%) was not a measure of FWT but only a difference in total UF between the two tests, due to UF through the SPs and the USPs;[13] so the difference in net UF of two PETs with different glucose concentrations is an indirect measure of OCG.[6]

The Double Mini-PET allowed one to assess directly the OCG, UFSP, and FWT. The slight underestimate of FWT, given by our method, supports the conclusion that in patients with UFF, the FWT and UFSP are both affected when the OCG is decreased. The finding of only a 50% decrease in net UF in the absence of sodium sieving[27] in aquaporin-1-deficient mice is another point that further supports this conclusion. According to our data, in PD patients with UFF, there were no differences or only slight increase in $MATC_{Creatinine}$ and $MTAC_{Glucose}$ values. All the patients with UFF had decreased OCG levels, normal or slight increased values of small solute diffusion and, owing to glucose, not decreased intraperitoneal osmotic pressure. Considering our data, the UFF could not be due to an increased peritoneal surface area (neoangiogenesis)[5] alone because, in that case, also the total area of pores, OCG, and then UF should have been increased.[6] In our study, the peritoneal UF, in the early phase, was reduced in the patients with UFF and no correlation was found between small peritoneal solute transport ($MATC_{Creatinine}$ and $MTAC_{Glucose}$) and OCG. Furthermore, in another study,[18] the patients with a high peritoneal transport showed no difference in endothelial aquaporin-1 expression in comparison with low transporters. However, this issue needs to be clarified evaluating the parameters of peritoneal membrane function in a large cohort of PD patients. Mateijsen *et al.*[28] in a histological study observed a time-related increase of neoangiogenesis in the peritoneal membrane in PD patients, whereas the recent study by Williams *et al.*[29] demonstrated a marked increase in the fibrosis observed in the sub-mesothelial compact zone and a marked increase in vasculopathy below the compact zone after several years of chronic exposure to PD fluids. In patients with UFF, it is possible that the association between the increasing abnormal microvasculature, produced by neoangiogenesis, and the fibrosis results in a decreased osmotic UF.[30]

The Double Mini-PET allows the simultaneous assessment of the OCG, FWT, and of the classical parameters of PET (D/D_0, $D/P_{Creatinine}$, $MTAC_{Glucose}$, $MTAC_{Creatinine}$, and D/P_{Na})[12] and it can be easily performed in whatever clinical context, because it does not require the use of intraperitoneal markers or complex computer calculations. These characteristics could facilitate the implementation of the evaluation of peritoneal membrane to a large PD patient's population and so could help to understand the mechanisms underlying the peritoneal membrane's failure.

Furthermore, the parameters obtained with the double Mini-PET can help the physicians in the correct prescription of PD therapy. For example, in the case of severe decrease of OCG, it is not useful to prescribe a higher glucose concentration in the PD solutions or a reduction of the time of the dwell, but it is more appropriate to prescribe an alternative osmotic agent like icodextrin; in the case of a 'normal' UF, OCG, and FWT, with a negative peritoneal UF during the long dwell (i.e., in CAPD), it is useful to prescribe a reduction of dwell time with the aid of automated PD.

Finally, the parameters obtained with the Double Mini-PET are easily applicable to the short time of automated PD therapy, which is becoming available worldwide.

In conclusion, the Double Mini-PET is a new, simple, and fast method to assess OCG, UFSP, and FWT, in addition to the classical parameters of PET. The Double Mini-PET could become a useful tool for periodical clinical evaluation of PD patients in everyday clinical practice. Further studies are required to determine the normal or pathological values of the classical parameters of the PET (UF, OCG, UFSP, and FWT) with this method.

MATERIALS AND METHODS

Patients

After having given their informed consent, 50 PD patients (20 males and 30 females with a mean age of 59 ± 14 years) were enrolled into the study and underwent the Double Mini-PET. The median time on PD was 5 months (3–21 months). Their condition was stable, and at the time of test, they had been free of peritonitis for at least 4 weeks. All patients used commercially available PD solutions. All patients had previously undergone a classical 3.86%-PET (4 h long), and the median time between the 3.86%-PET and the Double Mini-PET was 0 month (0–5 months). Four patients had shown UFF according to the results of the 3.86%-PET ($UF < 400$ ml).

Procedure

The first Mini-PET was performed with a 1.36% glucose solution and the second Mini-PET with a 3.86% glucose solution. The two solutions used in the Double Mini-PET were different only in glucose concentration (1.36 and 3.86%); all other solutes had the same concentrations. Each Mini-PET lasted 1 h, and they were performed consecutively in the same morning.

In all the cases, the dwell before the Double Mini-PET (the overnight dwell) was performed using a PD solution containing a glucose concentration of 1.36% with lactate as the buffer; the overnight solution was instilled at approximately 23:00 hours in the evening before the test and was drained at about 07:00 hours.

After the overnight drain, a fresh 2 l 1.36% glucose solution was infused in 10 min. The fresh PD fluid samples were taken from the bags at the end of the infusion. After the complete infusion of PD solution, and after having flushed back 30 ml of dialysate, 20 ml dialysate samples were taken after 1, 30, 60 min and after the complete dialysate collection by gravity for at least 20 min. After the 1.36% drain, a fresh 2 l 3.86% glucose solution was infused in 10 min and samples of fresh fluid and dialysate were taken with the same modality; the 3.86% dialysate was collected by gravity for at least 20 min as well. Blood samples were taken between the two tests.

The volume of the infused fresh PD solution and the drained dialysate was measured by weighing the bags and then subtracting the weight of the empty bags; no corrections were made for the differences in the specific weight of the solutions.

Analytical methods

Plasma and dialysate creatinine, total protein, and glucose concentrations were analyzed using a Hitachi 717 instrument (Hitachi, LTD, Tokyo, Japan); an enzymatic method was used to analyze creatinine to eliminate the effect of the high dialysate glucose concentration on the measurement of creatinine concentrations in the dialysate. The total sodium concentrations in the plasma, fresh PD solution, and dialysate were analyzed twice using an IL 943 flame photometer (Instrumentation Laboratory, Milan, Italy); the plasma-ionized sodium concentrations were analyzed using a direct ion-selective electrode (Stat Profile M, Nova Biomedical Corp., Waltham, MA, USA).

Calculations

D/D_0 was calculated dividing the dialysate glucose concentrations at the end of each Mini-PET by that of the fresh PD solution. $D/P_{Creatinine}$ and D/P_{Na} were calculated at the end of each Mini-PET;[31] the plasma water concentration of creatinine was considered.[32] $MTAC_{Creatinine}$ and $MTAC_{Glucose}$ were calculated by the simplified model of Garred[33] and to assess the MTAC during the test with 3.86% a value of $F = 0.5$ was used because of the high UF.[34]

The OCG is equal to the local hydraulic conductivity (L_p) multiplied by the membrane surface area (S) of pores and by the coefficient of glucose reflection (σ) ($L_pS\sigma$).[6,7]

Based on the principle of 'osmotic transients,'[19] it is possible to asses $L_pS\sigma$ (see Appendix for the calculations):

$$OCG = L_pS\sigma\,(\mathrm{ml/min/mmHg}) = \left[\frac{V_{3.86} - V_{1.36}}{19.3(G_{3.86} - G_{1.36})t}\right]1.7$$

where $V_{3.86}$ and $V_{1.36}$ are the volume (ml) of drained dialysate with 3.86 and 1.36% glucose solution, respectively, during the Double Mini-PET; 19.3 (mm Hg/mmol/l) is the product of absolute temperature and the gas constant at 37°C; $G_{3.86}$ and $G_{1.36}$ are the molar glucose concentrations (mmol/l) of the fresh PD solutions and were calculated as:

$$\mathrm{Glucose\,(mmol/l)} = \mathrm{glucose\,(mg/dl)}/18$$

t is the time of the dwells; in all Double Mini-PET, we considered the effective dwell time as the sum of the 60 min of dwell time, with a full fill volume (2 l), and the 50% of the time used for instillation and drainage; 1.7 was a correction factor to correct the dilution of glucose concentration, due to the peritoneal residual volumes, and to correct the differences in dialysate volumes between the 1.36% test and the 3.86% test that were assessed after 60 min (or more) and not at the beginning of the dwell.[6]

During the Double Mini-PET, the results of the 3.86%-Mini-PET were used to calculate the FWT as described previously:[13]

$$\mathrm{FWT\,(ml)} = \mathrm{Total\,UF\,(ml)} - \mathrm{UFSP\,(ml)}$$

During the 3.86%-Mini-PET, UFSP was calculated as follows:

$$\mathrm{UFSP\,(mL)} = [\mathrm{NaR\,(mmol)}1000]/\mathrm{Na_p\,(mmol/l)}$$

where NaR (mmol) was calculated as

$$[\mathrm{Volume_{DialysateOut}\,(L)Na_{DialysateOut}\,(mmol/l)}] - [\mathrm{Volume_{DialysateIn}\,(L)Na_{DialysateIn}\,(mmol/l)}]$$

and Na_p was the ionized sodium plasma water concentration assessed by direct ion-selective electrode.

Moreover, to correct the UFSP for the sodium diffusion, we calculated the NaR total (NaR_T) during the 1.36%-Mini-PET. This is because during a dwell with 1.36% glucose solution, the UF is low and therefore the sodium transport is mainly diffusive; however, we corrected the diffusive transport of sodium for the convective transport when the UF was positive. The NaR convective (NaR_C), during the 1.36%-Mini-PET, with approximation, was calculated as

$$\mathrm{NaR_C} = \mathrm{UF\,(L)Na_{DialysateOut}\,(mmol/l)}$$

The NaR diffusive (NaR_D), during the 1.36%-Mini-PET, was calculated as

$$\mathrm{NaR_D\,(mmol)} = \mathrm{NaR_T\,(mmol)} - \mathrm{NaR_C\,(mmol)}$$

Finally, we corrected the NaR (calculated during the 3.86%-Mini-PET) for sodium diffusion (calculated during the 1.36%-Mini-PET) as

$$\mathrm{NaR\,(corrected\,for\,sodium\,diffusion)\,(mmol)} = \mathrm{NaR\,(calculated\,during\,3.86\% - Mini - PET)\,(mmol)} - \mathrm{NaRD\,(calculated\,during\,1.36\% - Mini - PET)\,(mmol)}$$

The NaR corrected for sodium diffusion was used to correct UFSP and FWT for sodium diffusion.

Statistical analysis

The data with normal distribution were expressed as mean values ± 1 s.d. Median values and interquartile ranges were given for asymmetrically distributed data.

The same parameters of the two Mini-PETs (1.36%-Mini-PET and 3.86%-Mini-PET) during the Double Mini-PET were compared by t-test for paired data.

Mean values ± 1 s.d. of OCG were used to categorize PD patients, as reported elsewhere.[31]

Pearson linear correlation analysis was used to investigate possible relationships between OCG and $MTAC_{Creatinine}$, and between OCG and $MTAC_{Glucose}$. Furthermore, Pearson linear correlation analysis was used to investigate possible relationships between UF, during the classical 3.86%-PET, $MTAC_{Creatinine}$, and OCG; multiple linear correlation analysis was used to investigate the possible relationships between UF, during the classical 3.86%-PET, and $MTAC_{Creatinine}$ and OCG as covariates.

The parameters of the patients with and without UFF were compared by analysis of variance.

A $P<0.05$ was considered significant. All the statistical analyses were performed using JMP 4.0.0 statistical software (SAS Institute Inc., Cary, NC, USA).

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REFERENCES

1. Churchill DN, Thorpe KE, Nolph KD *et al.* Increased peritoneal membrane transport is associated with decreased patient and technique survival for continuous ambulatory peritoneal dialysis patients. *J Am Soc Nephrol* 1998; **9**: 1285–1292.
2. Brown EA, Davies SJ, Rutherford P *et al.* Survival of functionally anuric patients on automated peritoneal dialysis: the European APD Outcome Study. *J Am Soc Nephrol* 2003; **14**: 2948–2957.
3. Ates K, Nergizoglu G, Keven K *et al.* Effect of fluid and sodium removal in peritoneal dialysis patients. *Kidney Int* 2001; **60**: 767–776.
4. Heimburger O, Waniewski J, Werynski A *et al.* Peritoneal transport in CAPD patients with permanent loss of ultrafiltration capacity. *Kidney Int* 1990; **38**: 495–506.
5. Krediet RT, Lindholm B, Rippe B. Pathophysiology of peritoneal membrane failure. *Perit Dial Int* 2000; **20**(Suppl 4): S22–S42.
6. Rippe B, Venturoli D, Simonsen O *et al.* Fluid and electrolyte transport across the peritoneal membrane during CAPD according to the three-pore model. *Perit Dial Int* 2004; **24**: 10–27.
7. Stelin G, Rippe B. A phenomenological interpretation of the variation in dialysate volume with dwell time in CAPD. *Kidney Int* 1990; **38**: 465–472.
8. Rippe B, Stelin G. Simulation of peritoneal solute transport during CAPD. Application of two-pore formalism. *Kidney Int* 1989; **35**: 1234–1244.
9. Rippe B, Stelin G, Haraldsson B. Computer simulations of peritoneal fluid transport in CAPD. *Kidney Int* 1991; **40**: 315–325.
10. Rippe B. A three-pore model of peritoneal transport. *Perit Dial Int* 2000; **13**(Suppl 2): S35–S38.
11. Ho-Dac-Pannekeet MM, Schouten N, Langendijk MJ *et al.* Peritoneal transport characteristics with glucose polymer based dialysate. *Kidney Int* 1996; **50**: 979–986.
12. Carlsson O, Nielsen S, Zakaria El R, Rippe B. *In vivo* inhibition of transcellular water channels (aquaporin-1) during acute peritoneal dialysis in rats. *Am J Physiol* 1996; **271**: H2254–H2262.
13. La Milia V, Di Filippo S, Crepaldi M *et al.* Mini-peritoneal equilibration test: a simple and fast method to assess free water and small solute transport across the peritoneal membrane. *Kidney Int* 2005; **68**: 840–846.
14. Mujais S, Nolph KD, Gokal R *et al.* Evaluation and management of ultrafiltration problems in peritoneal dialysis. *Perit Dial Int* 2000; **20**(Suppl 4): S5–S21.
15. Waniewski J, Heimburger O, Werynski A *et al.* Osmotic conductance of the peritoneum in CAPD patients with permanent loss of ultrafiltration capacity. *Perit Dial Int* 1996; **16**: 488–496.
16. Smit W, Struijk DG, Ho-Dac-Pannekeet MM, Krediet RT. Quantification of free water transport in peritoneal dialysis. *Kidney Int* 2004; **66**: 849–854.
17. Venturoli D, Rippe B. Validation by computer simulation of two indirect methods for quantification of free water transport in peritoneal dialysis. *Perit Dial Int* 2005; **25**: 77–84.
18. Schoenicke G, Diamant R, Donner A *et al.* Histochemical distribution and expression of aquaporin-1 in the peritoneum of patients undergoing peritoneal dialysis: relation to peritoneal transport. *Am J Kidney Dis* 2004; **44**: 146–154.
19. Vargas FF, Johnson JA. An estimate of reflection coefficients for rabbit heart capillaries. *J Gen Physiol* 1964; **47**: 667–677.
20. Virga G, Amici G, da Rin G *et al.* Comparison of fast peritoneal equilibration tests with 1.36 and 3.86% dialysis solutions. *Blood Purif* 1994; **12**: 113–120.
21. Smit W, Langedijk MJ, Schouten N *et al.* A comparison between 1.36 and 3.86% glucose dialysis solution for the assessment of peritoneal membrane function. *Perit Dial Int* 2000; **20**: 734–741.
22. Pride ET, Gustafson J, Graham A *et al.* Comparison of a 2.5 and a 4.25% dextrose peritoneal equilibration test. *Perit Dial Int* 2002; **22**: 365–370.
23. Cara M, Virga G, Mastrosimone S *et al.* Comparison of peritoneal equilibration test with 2.27 and 3.86% glucose dialysis solution. *J Nephrol* 2005; **18**: 67–71.
24. Rippe B, de Arteaga J, Venturoli D. Aquaporins are unlikely to be affected in marked ultrafiltration failure: results from a computer simulation. *Perit Dial Int* 2001; **21**(Suppl): S30–S34.
25. Rippe B, Venturoli D. Simulations of osmotic ultrafiltration failure in CAPD using a serial three-pore membrane/fiber matrix model. *Am J Physiol Renal Physiol* 2007; **292**: F1035–F1043.
26. Monquil MCJ, Imholz ALT, Struijk DG *et al.* Does impaired transcellular water transport contribute to net ultrafiltration failure during CAPD? *Perit Dial Int* 1995; **15**: 42–48.
27. Ni J, Verbavatz JM, Rippe A *et al.* Aquaporin-1 plays an essential role in water permeability and ultrafiltration during peritoneal dialysis. *Kidney Int* 2006; **69**: 1518–1525.
28. Mateijsen MA, van der Wal AC, Hendriks PM *et al.* Vascular and interstitial changes in the peritoneum of CAPD patients with peritoneal sclerosis. *Perit Dial Int* 1999; **19**: 517–525.
29. Williams JD, Craig KJ, Topley N *et al.* Morphologic changes in the peritoneal membrane of patients with renal disease. *J Am Soc Nephrol* 2002; **13**: 470–479.
30. Flessner MF. The effect of fibrosis on peritoneal transport. In: Ronco C, Dell'Aquila R, Rodighiero MP (eds) *Peritoneal Dialysis: A Clinical Update*, vol. 150. Contrib Nephrol, Basel: Karger, 2006 pp 174–180.
31. Twardowski ZJ, Nolph KD, Khanna R *et al.* Peritoneal equilibration test. *Perit Dial Bull* 1987; **7**: 138–147.
32. Waniewski J, Heimburger O, Werynski A, Lindholm B. Aqueous solute concentrations and evaluation of mass transport coefficients in peritoneal dialysis. *Nephrol Dial Transplant* 1992; **7**: 50–56.
33. Garred LJ, Canaud B, Farrell PC. A simple kinetic model for assessing peritoneal mass transfer in chronic ambulatory peritoneal dialysis. *ASAIO J* 1983; **6**: 131–137.
34. Waniewski J, Werynski A, Heimburger O, Lindholm B. Simple models for description of small-solute transport in peritoneal dialysis. *Blood Purif* 1991; **9**: 129–141.

Appendix

Calculation of osmotic conductance of peritoneal membrane to glucose. To calculate the osmotic conductance of peritoneal membrane to glucose, we used the method previously described by Stelin and Rippe.[7]

During the peritoneal dialysis with glucose as osmotic agent the change rate that occurs in peritoneal volume in a given moment (dV/dt) is equal to net trans-peritoneal volume flow (J_V) and can be described by the following equation[6,7]:

$$\begin{aligned} dV/dt &= J_V \\ &= L_p S(\Delta P - \sigma_{prot}\Delta\pi_{pprot} + \sigma_g \Delta\pi_g \\ &\quad - \sum_{i=1}^{n} -\sigma_i \Delta\pi_i) - L \end{aligned} \tag{1}$$

where V, intraperitoneal volume; t, the time; L_P, the local hydraulic permeability; S the membrane surface area; ΔP the transcapillary hydrostatic pressure gradient; $\sigma_{prot}\Delta\pi_{prot}$ the transcapillary colloid osmotic pressure gradient exerted by protein; σ_g the average of osmotic reflection coefficient for glucose across the peritoneum; $\Delta\pi_g$ the ideal peritoneal crystalloid osmotic pressure difference exerted by glucose across a semi-permeable membrane; L the lymph flow;

$\sum_{i=1}^{n} -\sigma_i\Delta\pi_i =$ sum of all other effective crystalloid osmotic gradients acting across the peritoneal membrane.

The term $L_pS\sigma_g$ is the so-called osmotic conductance to glucose (OCG) of peritoneal membrane; it is a typical lumped parameter, which comprises at least two other

parameters that are not directly measured: hydraulic permeability of the membrane (L_pS) and the reflection coefficient for glucose (σ_g). The evaluation of these primary parameters is not possible in clinical settings.

Based on the principle of 'osmotic transient,'[19] it is possible to assess $L_pS\sigma_g$.

If the initial rate of J_V, due to osmosis, is assessed for two different glucose concentrations (e.g., 1.36 and 3.86%) and if the dwell time is shortened, for example, to 60 min and if all other parameters, excepts for the glucose concentration, remained constant between the two dwell, the differences in initial J_V (ΔJ_{V0}) can be described by[6,7]

$$\Delta J_{V0} = L_pS\sigma_g(\Delta\pi_{g2} - \Delta\pi_{g1}) \quad (2)$$

where $\Delta\pi_{g1}$ is the ideal (van't Hoff's) transperitoneal osmotic pressure gradient caused by the first glucose concentration and $\Delta\pi_{g2}$ that produced by the second glucose concentration. According to van't Hoff's law,

$$\Delta\pi_{g2} - \Delta\pi_{g1} = \Delta C\pi_{g2-1}RT \quad (3)$$

where RT is the product of absolute temperature and the gas constant (19.3 mm Hg/mmol/l) at 37°C and ΔC_{g2-1} is the molar difference between the initial glucose concentrations of the two solutions.

Assuming that the blood glucose concentration is constant between the two dwell, combining (2) and (3):

$$\Delta J_{V0} = L_pS\sigma_g RT(C_{g1} - C_{g2}) \quad (4)$$

Rearranging equation (4):

$$L_pS\sigma_g = \frac{\Delta J_{V0}}{RT(C_{g2} - C_{g1})} = \frac{V(60)_{3.86\%} - V(60)_{1.36\%}}{19.3(C_{g2} - C_{g1})} \quad (5)$$

By (5) it is possible to determine the osmotic conductance to glucose (OCG or $L_pS\sigma_g$). However, because we did not take into account the initial dilution of instilled glucose concentration with the residual volume and because we did not measure initial J_{V0} but the drained dialysate volume after 60 min of dwell, the value of osmotic conductance to glucose needs for a correction factor of 1.7.[6]

Calculation of free water transport across the peritoneal membrane. The calculation of free water transport across the peritoneal membrane is reported elsewhere.[13]